AAPS PharmSciTech, Vol. 15, No. 1, February 2014 (© 2013)
DOI: 10.1208/s12249-013-0045-z

Research Article

Microscopic Modeling of *País* Grape Seed Extract Absorption in the Small Intestine

Cristian Morales,[1] Marlene Roeckel,[1] and Katherina Fernández[1,2]

Received 1 July 2013; accepted 14 October 2013; published online 25 October 2013

Abstract. The concentration profiles and the absorbed fraction (F) of the *País* grape seed extract in the human small intestine were obtained using a microscopic model simulation that accounts for the extracts' dissolution and absorption. To apply this model, the physical and chemical parameters of the grape seed extract solubility (C_s), density (ρ), global mass transfer coefficient between the intestinal and blood content (k) (effective permeability), and diffusion coefficient (D) were experimentally evaluated. The diffusion coefficient ($D=3.45\times10^{-6}\pm5\times10^{-8}$ cm^2/s) was approximately on the same order of magnitude as the coefficients of the relevant constituents. These results were chemically validated to discover that only the compounds with low molecular weights diffused across the membrane (mainly the (+)-catechin and (−)-epicatechin compounds). The model demonstrated that for the *País* grape seed extract, the dissolution process would proceed at a faster rate than the convective process. In addition, the absorbed fraction was elevated (F=85.3%). The global mass transfer coefficient ($k=1.53\times10^{-4}\pm5\times10^{-6}$ cm/s) was a critical parameter in the absorption process, and minor changes drastically modified the prediction of the extract absorption. The simulation and experimental results show that the grape seed extract possesses the qualities of a potential phytodrug.

KEY WORDS: dose absorption; mathematical modeling; *País* grape seed extract; simulation.

INTRODUCTION

New drug development requires knowledge of both the drug's absorption in the human small intestine and the quantity of the drug that is absorbed into the bloodstream. These data are necessary before evaluating a compound for medical use in clinical trials. Many techniques can determine the absorbed fraction, including *in vivo* assays, which are necessary but raise ethical problems. The use of mathematical models has been utilized to predict the intestinal absorption. Thus, the simulation model may be used as a supplemental method in order to decide the dose to be tested in the clinical study. However, a subsequent *in vivo* determination is necessary.

The first model used to determine the absorbed fraction was based on a calculation of the drug concentration as a function of the pH (1), but the application of this model was limited to ionic drugs. Later, Dressman *et al.* (2) defined the absorption potential, which mainly evaluates the drug's physicochemical properties and cannot be used as the sole indicator of the drug bioavailability. Subsequently, Macheras and Symillides (3) determined the fraction dose (F) for orally administered compounds, i.e., the fraction of the dose that is absorbed. Sinko *et al.* (4) proposed an intestinal absorption model that considers the drug absorption using a global mass transfer coefficient between the small intestine and the bloodstream. However, the main problem of the aforementioned models is that they fail to describe the behavior of the nonionic species and to consider the dissolution or absorption processes. Thus, these models provide no information about the compound concentrations in the small intestine.

To overcome these problems, Oh *et al.* (5) proposed a microscopic model of intestinal absorption that considers the dissolution of a drug particle in the small intestine and the drug permeation from the small intestine into the bloodstream. Several dimensionless parameters have been defined to estimate F, including the absorption number (An), the dose number (Do), and the dissolution number (Dn), which has led to *a priori* information regarding the drug fate in the intestine.

Mathematical models have been rarely used to predict the intestinal absorption of the natural extracts. Although the study of natural bioactive compounds has increased over the past few decades, few studies in the field of phytopharmaceuticals have examined the absorption of these compounds.

Among these natural compounds, the *País* grape seed extract is distinguished for its high concentration of proanthocyanidins (PAs) and its capacity to decrease the *in vitro* angiotensin-converting enzyme (ACE) activity (6,7). In the human body, ACE inhibition decreases the arterial pressure (8); therefore, the *País* grape seed extract is an attractive, active source of natural compounds and could be used as a phytodrug for arterial hypertension. Nevertheless,

[1] Department of Chemical Engineering, University of Concepción, P.O. Box 160-C, Concepción, Chile.
[2] To whom correspondence should be addressed. (e-mail: kfernandeze@udec.cl)

the amount of the extract that can be absorbed in the small intestine based on the extract's physicochemical properties and physiological parameters has not yet been determined.

The objective of this study was to obtain and analyze the concentration profiles and the absorbed fraction (F) of the *País* grape seed extract in the human small intestine using a microscopic model that accounts for the dissolution and the absorption. To apply this model, the following physical and chemical parameters of the extract were experimentally determined: the solubility (C_s), the density (ρ), the global mass transfer coefficient between the intestinal and the blood content (k) (effective permeability), and the diffusion coefficient (D).

MATERIALS AND METHODS

Materials

Sodium acetate, acetone, acetonitrile, acetic acid, hydrochloric acid, orthophosphoric acid, sodium carbonate, potassium chloride, ammonium phosphate, methanol, *n*-hexane, and ammonium sulfate were supplied by Merck (Darmstadt, Germany). Additionally, (+)-catechin, (−)-epicatechin, phloroglucinol, and the Folin–Ciocalteu reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). A Millipore Milli-Q (Bedford, MA, USA) water purification system was used to purify the water used in all solutions.

País Grape Seed Extract Production

The *País* véraison grapes were collected on March 15, 2012, from the Quillón Valley, Biobío Region, Chile, and the grapes were preserved in sealed bags and frozen at −18°C. The seeds of the 200 *País* grapes were separated manually. The extraction was performed in an Erlenmeyer flask with 250 mL of 33% (*v*/*v*) of acetone in water using on a New Brunswick G24 gyratory shaker (New Brunswick Scientific Co., Edison, NJ, USA) for 15 h at room temperature and in the dark to avoid oxidation. The acetone was eliminated at a reduced pressure and temperature (<35°C) in a rotary evaporator (Bibby Sterilin Ltd, RE-100B, Stone Staffordshire, England) until 50 mL of the grape seed extract remained. The liposoluble compounds of the grape seed extract were removed using three 50-mL washing steps with *n*-hexane. Mass transfer coefficients, diffusion coefficient, and phenol concentration were determined with this raw extract. For the solubility and density determination, the extract was frozen at −18°C, lyophilized using a Labconco Freezone 6 freeze dryer (Labconco Corporation, Kansas City, MO, USA), and stored in a dark place until use.

Density and Solubility Determination of the *País* Grape Seed Extract

The density was determined as previously described by Jin *et al.* (9). A test tube was gauged by striking it ten times against a flat surface, and the mass and volume were measured.

The extract solubility was determined as previously described (10). The grape seed extract was dissolved in 10 mL of the Milli-Q water until the saturation point was reached. The solubilization time for all components of the extract was about 3 to 5 s. Manual agitation was performed for 30 min. Then, the mixture was centrifuged at 5,000×g for 10 min (Heraeus, Biofuge Primo, Germany). The supernatant was dried at 105°C until the weight remained constant.

The Global Mass Transfer Coefficient Determination of the *País* Grape Seed Extract

The global mass transfer coefficient (effective permeability) of the *País* grape seed extract between the intestinal and blood content was determined using a Franz cell chamber. The intestinal wall was simulated using an acetate cellulose membrane with a molecular weight cutoff of 12 kDa (Spectrum Labs, Spectra/Por 2, USA), allowing only the low molecular weight compounds to permeate into the acceptor compartment. This pore diameter has been utilized to study previously gastric and intestinal simulations (11,12). The cell was comprised of two compartments: an upper compartment with a volume of 5 mL and a lower compartment with a volume of 12 mL. The raw grape seed extract was placed in the upper compartment, and the lower compartment contained Milli-Q water. Aliquots of 0.15 mL were withdrawn at 30, 60, 90, 120, 150, and 180 min after the grape seed extract was placed in the upper compartment; these aliquots were stored in Eppendorf tubes at 4°C to determine the total concentration of the phenols. The effective permeability was calculated according to Eq. (1) (13):

$$k = \frac{\mathrm{dC}}{\mathrm{dt}} \frac{V_i}{A_i C_{cs}} \tag{1}$$

where A_i is the cross-sectional area (square centimeter) of the Franz cell, C_{cs} is the initial concentration (milligram equivalent catechin per cubic centimeter) in the upper compartment, C is the concentration (milligram equivalent catechin per cubic centimeter) in the lower compartment, k is the global mass transfer coefficient (centimeters per second) of the drug between the upper and lower compartment, t is the time (second), V_i is the lower compartment volume (cubic centimeter) of the Franz cell, and dC/dt is the slope (grams per cubic centime per second) of the graph C *vs*. t.

Determination of the Grape Seed Extract Diffusion Coefficient (D)

The diffusion coefficient was evaluated with a diaphragm cell, as previously described (14). Briefly, the cell consisted of two compartments with a 35-mL usable volume in each compartment. At 70 rpm, the upper compartment was agitated using a helix, and the lower compartment was agitated using a magnetic stirrer. Both compartments were separated by an acetate cellulose membrane with a molecular weight cutoff of 12 kDa (Spectrum Labs, Spectra/Por 2, USA). The cell was placed in a thermostatic bath at 37°C. The lower compartment contained 35 mL of Milli-Q water, and the upper compartment contained 35 mL of the raw grape seed extract. After 180 min, both compartments were emptied, and the total concentration of the phenols and the PAs were

determined. The diffusion coefficient was calculated using Eq. (2) (15):

$$D = \frac{1}{\mathrm{Bt}} \ln\left[\frac{C_{1.0} - C_{2.0}}{C_1 - C_2}\right] \tag{2}$$

where B is the cell constant (cm^{-2}), $C_{1.0}$ is the initial concentration in the upper compartment, $C_{2.0}$ is the initial concentration in the lower compartment, C_1 is the concentration at the time t in the upper compartment, and C_2 is the concentration at the time t in the lower compartment. All concentrations are in units of milligram equivalent catechin per cubic centimeter, and t is the time in second.

The cell was calibrated with 0.1 mol/L KCl, resulting in a cell constant (B) value of 8.96±0.05 cm^{-2}. The measurements of the KCl diffusion coefficient were performed at 25°C at concentrations of 0.01, 0.1, 0.2, 0.5, and 1 mol/L, and the results were compared to the values reported in the literature (15) and showed a relative error of less than 8%.

Determination of the Total Concentration of the Phenols

The total phenolic content of each sample was analyzed following the procedure reported by Jerez *et al.* (16). First, 2.5 mL of the Folin–Ciocalteu reagent was diluted with 2 mL of a 7.5% (w/v) sodium carbonate solution and 0.5 mL of the sample. Then, the solution was heated for 13 min at 45°C, and the solution's absorbance was measured at 765 nm (Techcomp spectrophotometer, UV-2300, China). The total concentration of the phenols was expressed in units of milligram equivalent catechin per cubic centimeter using Eq. (3):

$$F_t = f_c \cdot A_{bs} \cdot D_i \tag{3}$$

where A_{bs} is the absorbance (nanometer) of the sample, D_i is the sample dissolution (in this case, it is 10), f_c is an equivalence factor (79.34 mg eq catechin/cm^3 nm) and F_t is the total concentration of the phenols (milligram equivalent catechin per cubic centimeter).

Determination of the PAs from the *País* Grape Seed Extract

The samples from Franz cell were analyzed by phloroglucinolysis. An acid catalysis with phloroglucinol was completed to determine the chemical composition following the procedure established by Kennedy and Jones (17), using the modifications described by Cerpa-Calderón and Kennedy (18). A 0.5-mL aqueous sample was dried and then diluted in 0.5 mL of methanol. This solution was reacted with 0.1 mol/L HCl in methanol and 100 g/L of phloroglucinol for 20 min at 50°C. The reaction was stopped by adding five volumes of 0.04 mol/L of sodium acetate. Finally, the resulting adducts and monomers were analyzed by HPLC-RP (Young Lin, ACME 9000, Korea).

Predictive Mathematical Modeling for the *País* Grape Seed Extract Absorption

The microscopic modeling previously established by Oh *et al.* (5) is presented in Eqs. (4) and (5):

$$\frac{dr^*}{dz^*} = -\frac{Dn}{3} \cdot \frac{1 - C^*}{r^*} \tag{4}$$

$$\frac{dC^*}{dz^*} = Dn \cdot Do \cdot r^* (1 - C^*) - 2An \cdot C* \tag{5}$$

where

$$An = \frac{k\pi RL}{Q} = \frac{\text{radial absorption rate}}{\text{axial convection rate}} \tag{6}$$

$$Do = \frac{M_0/V_0}{C_s} = \frac{\text{dose concentration}}{\text{solubility}} \tag{7}$$

$$Dn = \frac{(D/r_0)C_s\left(4\pi r_0^2\right)/\left((4/3)\pi r_0^3 \rho\right)}{Q/\left(\pi R^2 L\right)} = \frac{\text{residence time}}{\text{dissolution time}} \tag{8}$$

In Eqs. (4)–(8), C^* is the dimensionless concentration (C_b/C_s) along the intestine, z^* is the dimensionless length of the intestine (z/L), r^* is the dimensionless particle radius (r_p/r_0), An is the drug absorption number, Do is the dose number, Dn is the dissolution number, Q is the volumetric flow rate in the intestinal membrane, r_0 is the particle initial radius, r_p is the particle radius at the position z, z is the axial coordinate of the intestine, L and R are the length and radius of the intestinal membrane, respectively, k is the global mass transfer coefficient, M_0 is the administered dose, V_o is the intestinal volume, C_s is the drug saturation concentration, C_b is the concentration at position z, D is the drug diffusion coefficient, and ρ is the drug density.

The resolution of this equation system was determined using the MATLAB® software (Mathworks, r2010a, USA) with the command ODE13S, which applies a variable step Runge–Kutta routine for the stiff equations. The *País* grape seed extract was assumed to enter the small intestine as a solid, spherical particle that was not dissolved in the stomach, with a mean radius of 25 μm (5) and 0.1 g of the grape seed extract. Using the predicted concentration profile of the grape seed extract in the small intestine, the absorbed fraction of the extract (F) and the dimensionless factors Dn, An, and Do were determined.

Sensitivity Analysis of the Experimental Parameters

The model sensitivity was evaluated against the experimentally determined parameters; thus, the density,

solubility, diffusion coefficient, and effective permeability varied between 0.1 and 2 times their baseline values, and the C^*, r^*, and F were then evaluated for each set of parameters. In particular, the density was varied to 0.1, 0.5, and 1 g/mL, which represents a doubling of the experimental value, the commercial extract value, and the water density.

Statistical Analysis

All experimental design analyses were performed in duplicate. The spectrophotometric determinations were performed in triplicate, and the arithmetic means were expressed with the standard deviation using Microsoft Excel®.

RESULTS AND DISCUSSION

País Grape Seed Extract Density and Solubility

The evaluation of the density and solubility of the grape seed extract's phenolic compounds is necessary for an adequate design of the processes and products. The *País* grape seed extract density (ρ) was measured at 45.89±0.05 g/L, ten times less than the density of the other commercial extracts from the *Vitis vinifera* grape seeds, which have densities ranging from 0.4 to 0.5 g/mL (Sinoway Industrial Co. Ltd, China). The mean drug density is approximately 1 g/mL (5,19), which also exceeded the values determined in this study. This discrepancy can be explained by the different drying processes used. The commercial extract was spray dried, whereas the extract in this study was lyophilized. Gallo *et al.* (20) claimed that with spray drying, the density increases because the resultant extracts are finely divided. The low density of an extract can be detrimental to the drug design; large volumes could be needed for a therapeutic dose and to compress or concentrate the extract to ensure an adequate drug delivery volume. Others authors have used densities from 70 to 280 g/L of epigallocatechin-3-*O*-methylgallate, with doses from 10 to 40 mg/kg *in vivo* experiments showing a strong effect on blood pressure (21), which was in the range of our simulation study. How the extract density changes influence the intestinal absorption process will be modeled in the next section.

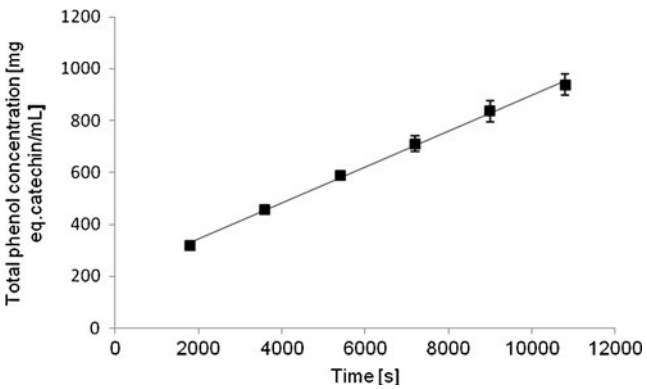

Fig. 1. Determination of the concentration in the lower compartment of the Franz cell used to determine the global mass transfer coefficient for *País* grape seed extract

Table I. Comparison of Global Mass Transfer Coefficients Between the Intestinal Content and Bloodstream (Permeability Coefficient) for Drugs Obtained with Single-Pass Intestinal Perfusion in Rats (22) and the Value for the *País* Grape Seed Extract

Drug	Global mass transfer coefficient, $k\times10^{-4}$ [cm/s]
Propranolol	2.90
Metopropol	1.20
Atenolol	0.12
País grape seed extract	1.53

The solubility (C_s=38.15±1 mg/mL) of the *País* grape seed extract was larger than both the solubility (C_s=5.03 mg/mL) of the synthetic (+)-catechin (the most common flavan-3-ols) compound and the solubility of the other natural phenolic compounds found in grapes (22). The high extract solubility may be due to the presence of the hydroxyl groups, which favors the formation of hydrogen bonds with the water. In addition, freeze-dried products often show fast dissolution, which may be even more important for absorption than the overall saturation solubility.

The Effective Permeability (k)

The experimentally determined value for the global mass transfer coefficient (k) between the upper and lower compartment of the Franz cell was $k=1.53\times10^{-4}\pm5\times10^{-6}$ cm/s. Figure 1 shows that the total phenol concentration in the lower compartment of the Franz cell changed linearly with time, which is consistent with the relationship shown in Eq. (1). Table I compares the k value *in vitro* obtained in this study to the k values obtained for other compounds (23). These values were obtained through *in vivo* rat experiments and were subsequently correlated to the human small intestine. *In vivo* mass transfer data is scarce for drugs obtained from natural compounds. Ideally, classification of drug permeability would be based on experimental human jejunal permeability data. However, since such information is readily available for only a small fraction of drugs, well-defined mass balance studies can be an alternative for drug parameter estimations.

From Table I, comparison of the value of the effective extract permeability is within the range of the drugs' value used for the treatment of several diseases of the cardiovascular system, especially hypertension (propanolol, metopropol, and atenolol) (23).

Recently, Tian *et al.* (24) and Ou *et al.* (25) experimentally determined the global mass transfer coefficients for the

Table II. The Mean Degree of Polymerization of the *País* Grape Seed Extract at the Different Permeation Times Obtained from the Franz Cell

Time [min]	mDP
90	1.000±0.000
135	1.175±0.061
180	1.150±0.051

mDP mean degree of polymerization

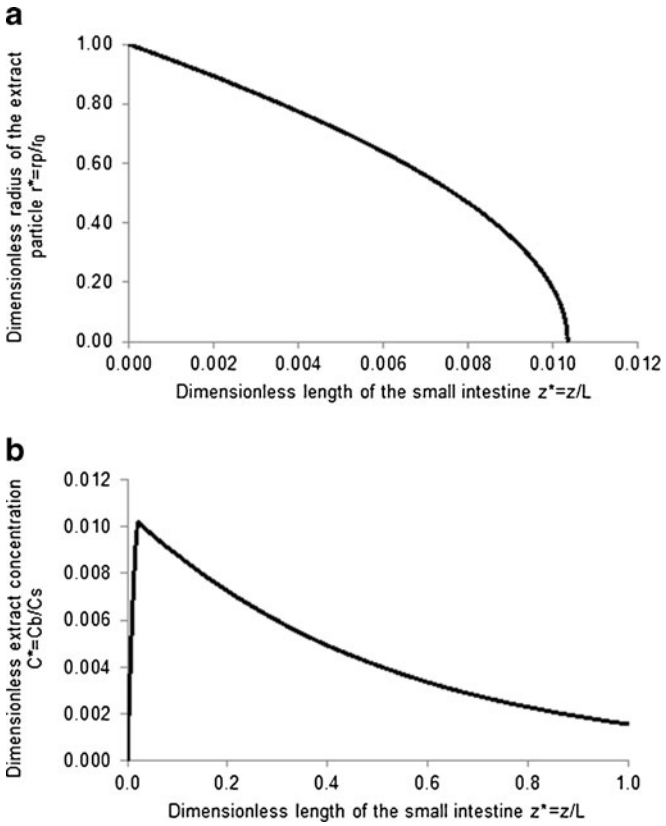

Fig. 2. **a** The dimensionless particle radius (r^*) of the *País* grape seed extract in the small intestine, as determined by the model. **b** The dimensionless concentration profile (C^*) of the *País* grape seed extract in the small intestine, as determined by the model

commercial (−)-epicatechin and (+)-catechin compounds at $k=0.6\times10^{-6}\pm0.05\times10^{-6}$ and $k=0.7\times10^{-6}\pm0.05\times10^{-6}$ cm/s, respectively. Both values are lower than those values determined in our study. On one hand, both compounds, (−)-epicatechin and (+)-catechin, were pure and synthetically obtained (24,25), whereas the grape seed extract is a natural and complex material. On the other hand, these authors used a Caco-2 cell monolayer to model the biological membranes, whereas we used an acetate cellulose membrane.

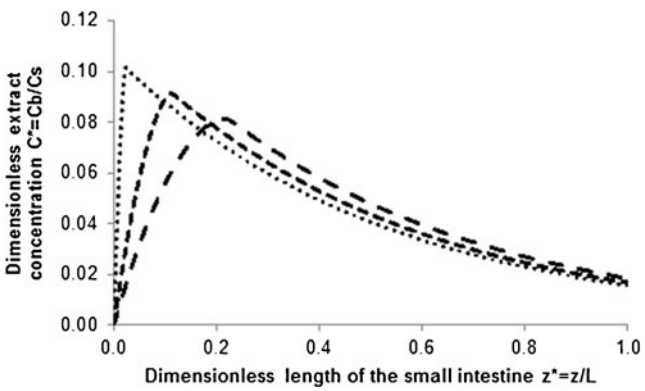

Fig. 3. The influence of the density on the dimensionless concentration profiles (C^*) of the *País* grape seed extract in the small intestine, as predicted by the model; 0.1 g/mL (*dotted line*), 0.5 g/mL (*dashed line*), and 1 g/mL (*dashed dotted line*)

Diffusion Coefficient of *País* Grape Seed Extract

The diffusion coefficient (D) of the *País* grape seed extract was $3.45\times10^{-6}\pm5\times10^{-8}$ cm^2/s at 37°C. Usually for aqueous solutions, the D is approximately 10^{-5} cm^2/s and is typically lower for the macromolecules (26), which could explain the relatively low value determined in this study. Srinivas *et al.* (27) determined the diffusion coefficients at 39°C for the (−)-epicatechin and (+)-catechin compounds at 2.66×10^{-6} and 3.07×10^{-6} cm^2/s, respectively. Thus, the obtained D was on the same order of magnitude as the coefficients of the relevant constituents.

Chemical Characterization of the *País* Grape Seed Extract

The studied PAs were characterized by their mean degree of polymerization (mDP) and their structure conformation (the extension and terminal compounds). The original extract had an mDP=6.53 (average number of units in the polymer chain) with the following proportional molar composition (in moles): 0.22 (+)-catechin-phloroglucinol, 0.58 (+)-epicatechin-phloroglucinol (EC-P), 0.07 (+)-catechin (C), 0.04 epicatechin-3-*O*-gallate-phloroglucinol, 0.07 (−)-epicatechin (EC), and 0.01 epigallocatechin. Based on the acid catalytic phloroglucinol reaction, the proanthocyanidin yield was about 90%. This means that 10% are not phenolic compounds which were not hydrolyzed or did not react with phloroglucinol (17).

Table III. The Influence of the Density of the *País* Grape Seed Extract on the Dissolution Number and the Absorbed Fraction, as Predicted by the Model

Density [g/mL]	Dissolution number	Absorbed fraction [%]
0.045	154	85.3
0.100	69	85.2
0.500	14	84.1
1.000	7	82.5

Dn dissolution number, *F* absorbed fraction

In the diffusion assays, the compounds that permeated through the membrane had an mDP close to 1 (Table II), and the compounds had the following proportional molar masses (in moles): 0.49 C, 0.39 EC, and 0.12 EC-P. Thus, only the compounds of a low molecular weight diffused passively across the membrane, which would be favorable for the grape seed extract bioavailability. As it has been suggested, EC dimers and monomers are absorbed in the small intestine (28); this membrane could be predictive of *in vivo* absorption.

Microscopic Modeling of the *País* Grape Seed Extract Absorption in the Small Intestine

When modeling, the grape seed extract was assumed to be administered by an oral capsule, which contained spherical particles of the extract with a mean radius of 25 μm (5) and a 0.1-g dose. Figure 2a shows the variation of the extract particle radius (r^*) along the small intestine membrane (z^*). Due to the high solubility of the grape seed extract, the extract particles were completely dissolved. The process dissolution period was smaller than the time period required for the extract absorption, i.e., the dissolution was faster than the absorption of the extract. Therefore, the solubility was not a limiting step in the whole dissolution–absorption process. The small particle size also benefited the mass transfer from the particles to the intestinal medium because of the increased particle surface-to-volume ratios.

Figure 2b shows the concentration variation of the *País* grape seed extract in the small intestine (C^*) with the length of the intestine (z^*). The maximum concentration observed corresponds to 1% of the grape seed extract's solubility and appears simultaneously with the total particle solubilization. Therefore, the simulation predicts that the particle dissolution process would occur faster than the particle convective process in the intestinal medium. Additionally, the concentration (C^*) never reached zero, which indicates that a fraction of the grape seed extract was not absorbed in the small intestine and agrees with the other human experimental studies showing that the absorbed fraction is less than 1 (28,29).

The dissolution number (Dn) was 154, and the active compound residence time in the small intestine predicted by the model was greater than the particle dissolution time, which could promote the absorption and subsequent particle availability in the blood. In this study, the absorption number (An) was 1.07; this value indicates that the extract absorption rate was equivalent to the axial velocity in the small intestine, which produces the concentration soft decay curve with an equal convective transport and absorption rate. The dose number (Do) was 0.01 after a dose of 0.1 g. This dose number indicates that the maximum extract concentration in the small intestine was less than the solubility. Oh *et al.* (5) and Amidon *et al.* (19) claimed that a low Do enhances the extract absorption.

Finally, based on the intestinal mass balance, the predicted absorbed F was 85.3%. Thus, this result suggests that the *País* grape seed extract would be well absorbed in the human small intestine. These results support other evidence showing that the tannins present in food are absorbed in the human small intestine (28,29). However, despite the model's robustness and physical representation, it is limited by the steady-state assumption, which can vary with the drug administration procedures.

Experimental Parameter Sensitivity Analysis

Because the extract ρ was lower than those of the traditional drugs and because ρ is a modifiable parameter during the extract production, its influence on the C^*, r^*, Dn, and F was determined by the simulation. For an increased ρ, the maximum extract concentration (C^*) in the small intestine decreased (Fig. 3), which resulted in a lower concentration gradient between the intestine and the blood and caused a lower mass

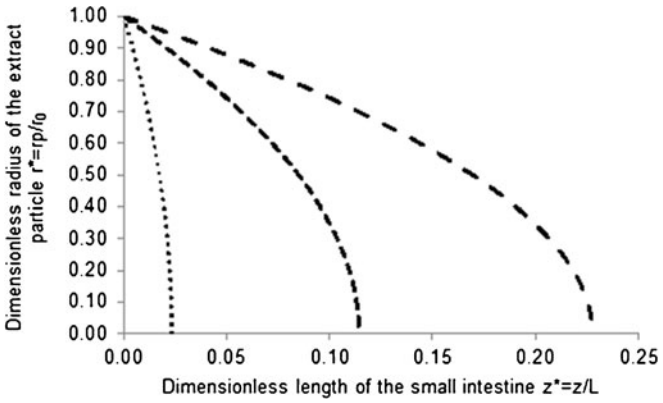

Fig. 4. The influence of the density on the dimensionless radius (r^*) of the *País* grape seed extract particles along the intestinal membrane, as predicted by the model; 0.1 g/mL (*dotted line*), 0.5 g/mL (*dashed line*), and 1 g/mL (*dashed dotted line*)

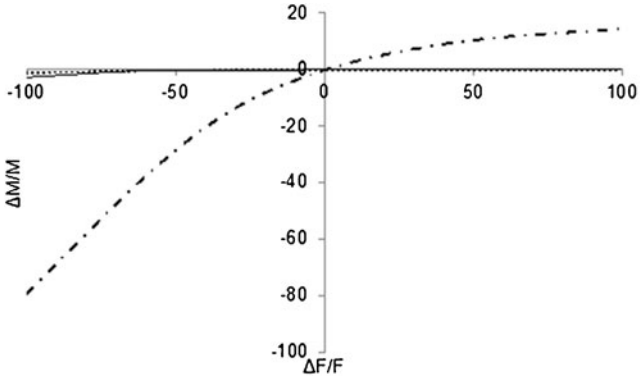

Fig. 5. The sensitivity analysis of the absorbed fraction (F) for the different parameter values (M) of the model. The baseline values for the M are $3.815\times10^{-2}\pm1\times10^{-4}$ g/mL for the solubility (*straight line*); $1.53\times10^{-4}\pm5\times10^{-6}$ cm/s for the global coefficient of mass transfer (*dashed dotted line*); and $3.45\times10^{-6}\pm5\times10^{-8}$ cm^2/s for the diffusion coefficient (*dotted line*)

flux according to Fick's law. These results explain why the increase in density from 0.1 to 1 g/mL decreased the F by 3%, as shown in Table III. Therefore, the extract density could be increased until a suitable volume could be orally administered because the absorbed extract fraction does not decrease significantly. With a larger ρ, the particles' movement increased without an increased dissolution because a greater mass was contained in the same volume (Fig. 4) and because the Dn was inversely proportional to the density (Table III). Thus, the dissolution time increased and nearly approached the extract residence time.

Along with the previous observations, a simulation was executed to determine the effect of decreasing the k to 10^{-5} and 10^{-6} cm^2/s on the F and showed that the F was reduced to 17.82% and 2.44%, respectively. The mass transfer rate from the small intestine to the blood decreased, predominantly due to the transport through the small intestine, because the intestine wall provided some resistance.

When the D and C_s were modified, a minimal variation in F was observed: only 2.75% and 1.08%, respectively (Fig. 5). A major change occurred when k was modified, which generated a maximum variation of −79.12%. Thus, small variations in k produced very large changes in F, which suggests that k is the most important influencing factor in the human small intestine absorption process when solubility is good and dissolution is fast.

CONCLUSIONS

Both the physical and chemical parameters of the *País* grape seed extract were experimentally evaluated. The *País* grape seed extract absorption in the human small intestine was successfully modeled using a mathematical model to describe the extract dissolution and the permeation from the intestine into the bloodstream; thus, an estimate of the absorbed fraction of the grape seed extract dose was obtained. This model demonstrated that the time required for the *País* grape seed extract to dissolve corresponded to the time required for the extract particles to traverse less than one one-hundredth of the total intestinal length and that the permeation resulted in a high absorbed F (85.3%). The global mass transfer coefficient (k) was a critical parameter in the absorption process because minor changes in k drastically modified the F value. Therefore, the current modeling suggests that k should be experimentally determined using biological membranes to improve the overall model precision.

ACKNOWLEDGMENTS

The authors thank the FONDECYT, Chile for the financial support of this investigation, project nos. 11080001 and 1120148.

REFERENCES

1. Waterbeemd H, Testa B. Drug bioavailability. Weinheim: Wiley; 2009.
2. Dressman JB, Amidon GL, Fleisher D. Absorption potential: estimating the fraction absorbed for orally administered compounds. J Pharm Sci. 1985;74(5):588–9.
3. Macheras PE, Symillides MY. Toward a quantitative approach for the prediction of the fraction of dose absorbed using the absorption potential concept. Biopharm Drug Dispos. 1989;10(1):43–53. doi:10.1002/bdd.2510100106.
4. Sinko PJ, Leesman GD, Amidon GL. Predicting fraction dose absorbed in humans using a macroscopic mass balance approach. Pharm Res. 1991;8(8):979–88. doi:10.1023/a:1015892621261.
5. Oh DM, Curl RL, Amidon GL. Estimating the fraction dose absorbed from suspensions of poorly soluble compounds in humans: a mathematical model. Pharm Res. 1993;10(2):264–70. doi:10.1023/a:1018947113238.
6. Eriz G, Sanhueza V, Roeckel M, Fernández K. Inhibition of the angiotensin-converting enzyme by grape seed and skin proanthocyanidins extracted from *Vitis vinífera* L. cv. *País*. LWT Food Sci Technol. 2011;44:860–5.
7. Godoy S, Roeckel M, Fernandez K. Influence of the structure and composition of the Pais grape proanthocyanidins on the inhibition of angiotensin I-converting enzyme (ACE). Food Chem. 2012;134(1):346–50. doi:10.1016/j.foodchem.2012.02.171.
8. Scribner AW, Loscalzo J, Napoli C. The effect of angiotensin-converting enzyme inhibition on endothelial function and oxidant stress. Eur J Pharmacol. 2003;482(1–3):95–9. doi:10.1016/j.ejphar.2003.10.002.
9. Jin P, Madieh S, Augsburger LL. Selected physical and chemical properties of Feverfew (*Tanacetum parthenium*) extracts important for formulated product quality and performance. AAPS PharmSciTech. 2008;9(1):22–30. doi:10.1208/s12249-007-9017-5.
10. Belscak-Cvitanovic A, Benkovic M, Komes D, Bauman I, Horzic D, Dujmic F, *et al*. Physical properties and bioactive constituents of powdered mixtures and drinks prepared with cocoa and various sweeteners. J Agric Food Chem. 2010;58(12):7187–95. doi:10.1021/jf1005484.
11. Tharakan A, Norton IT, Fryer PJ, Bakalis S. Mass transfer and nutrient absorption in a simulated model of small intestine. J Food Sci. 2010;75(6):E339–46. doi:10.1111/j.1750-3841.2010.01659.x.
12. McDougall GJ, Fyffe S, Dobson P, Stewart D. Anthocyanins from red wine—their stability under simulated gastrointestinal digestion. Phytochemistry. 2005;66(21):2540–8.
13. Kumar KV, Karnati S, Reddy M. Caco-2 cell lines in drug discovery—an updated perspective. J Basic Clin Pharm. 2010;1(2):63–6.
14. Wu Y, Ma P, Liu Y, Li S. Diffusion coefficients of l-proline, l-threonine and l-arginine in aqueous solutions at 25°C. Fluid Phase Equilib. 2001;186(1–2):27–38. doi:10.1016/s0378-3812(01)00355-7.
15. Lobo VMM, Ribeiro ACF, Verissimo LMP. Diffusion coefficients in aqueous solutions of potassium chloride at high and low concentrations. J Mol Liq. 1998;78(1–2):139–49. doi:10.1016/s0167-7322(98)00088-9.

16. Jerez M, Pinelo M, Sineiro J, Nunez MJ. Influence of extraction conditions on phenolic yields from pine bark: assessment of procyanidins polymerization degree by thiolysis. Food Chem. 2006;94(3):406–14.
17. Kennedy JA, Jones GP. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. J Agric Food Chem. 2001;49(4):1740–6.
18. Cerpa-Calderon FK, Kennedy JA. Berry integrity and extraction of skin and seed proanthocyanidins during red wine fermentation. J Agric Food Chem. 2008;56(19):9006–14. doi:10.1021/jf801384v.
19. Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res. 1995;12(3):413–20. doi:10.1023/a:1016212804288.
20. Gallo L, Llabot JM, Allemandi D, Bucalá V, Piña J. Influence of spray-drying operating conditions on *Rhamnus purshiana* (Cáscara sagrada) extract powder physical properties. Powder Technol. 2011;208(1):205–14. doi:10.1016/j.powtec.2010.12.021.
21. Liu JC, Hsu FL, Tsai JC, Chan P, Ya-Hsin J, Liu G, *et al*. Antihypertensive effects of tannins isolated from traditional Chinese herbs as non-specific inhibitors of angiotensin converting enzyme. Life Sci. 2003;73(12):1543–55. doi:10.1016/S0024-3205(03)00481-8.
22. Mota FL, Queimada AJ, Pinho SP, Macedo EA. Aqueous solubility of some natural phenolic compounds. Ind Eng Chem Res. 2008;47(15):5182–9. doi:10.1021/ie071452o.
23. Zakeri-Milani P, Valizadeh H, Tajerzadeh H, Azarmi Y, Islambolchilar Z, Barzegar S, *et al*. Predicting human intestinal permeability using single-pass intestinal perfusion in rat. J Pharm Pharm Sci. 2007;10(3):368–79.
24. Tian XJ, Yang XW, Yang X, Wang K. Studies of intestinal permeability of 36 flavonoids using Caco-2 cell monolayer model. Int J Pharm. 2009;367(1–2):58–64. doi:10.1016/j.ijpharm.2008.09.023.
25. Ou K, Percival SS, Zou T, Khoo C, Gu L. Transport of cranberry A-type procyanidin dimers, trimers, and tetramers across monolayers of human intestinal epithelial Caco-2 cells. J Agric Food Chem. 2012;60(6):1390–6. doi:10.1021/jf2040912.
26. Cussler EL. Diffusion: mass transfer in fluid system. 2nd ed. Cambridge: Cambridge University Press; 2007.
27. Srinivas K, King JW, Howard LR, Monrad JK. Binary diffusion coefficients of phenolic compounds in subcritical water using a chromatographic peak broadening technique. Fluid Phase Equilib. 2011;301(2):234–43. doi:10.1016/j.fluid.2010.12.003.
28. Yang C, Sang S, Lambert J, Lee MJ. Bioavailability issues in studying the health effects of plant polyphenolic compounds. Mol Nutr Food Res. 2008;52(1):S139–51.
29. Lee MJ, Wang ZY, Li H, Chen L, Sun Y, Gobbo S, *et al*. Analysis of plasma and urinary tea polyphenols in human subjects. Cancer Epidemiol Biomarkers Prev: Publ Am Assoc Cancer Res cosponsored by the Am Soc Prev Oncol. 1995;4(4):393–9